

BROMINATED UNSATURATED ACIDS FROM THE MARINE SPONGE XESTOSPONGIA SP.

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Abstract - Six brominated unsaturated polyenyne C₉, C₁₆ and C₁₈-acids have been isolated from the marine sponge Xestospongia sp., collected in the straits of the Gulf of Eilat (the Red Sea), and their structure elucidated by spectroscopic methods. The ¹H and ¹³C resonance lines of four compounds were fully assigned by means of homonuclear shift-correlation 2D-NMR (COSY-45) experiments and in one case also by heteronuclear CH-correlation spectra.

A recent publication on the isolation of 18-bromooctadeca-9(E),17(E)-dien-7,15-diyneic acid from the sponge Xestospongia testudinaria (collected near Townsville, Australia)¹ brought us to report on our findings of six brominated polyenyne acids from a Xestospongia sp. collected in the straits of the Gulf of Eilat (the Red Sea).

Flash chromatography of the petroleum ether and dichloromethane extracts of the freeze-dried sponge resulted in an acidic fraction which was methylated with CH₂N₂ to give the corresponding methyl esters. HPLC of the latter methyl esters on a porasil A column eluted with cyclohexane/ethylacetate (9:1) revealed at least sixteen compounds out of which we purified six brominated unsaturated esters (1b-6b). The major compounds (1, 3 and 6) could also be isolated directly as the free acids by repeated chromatographies of the crude extract on Sephadex LH-20 and silica gel columns.

Characteristic for all compounds, except for one (6), were acetylene absorptions in the IR-spectrum (around 2200 cm⁻¹) and the multi-coupled resonance lines of vinyl protons sited on conjugated double bonds in the 5.5-6.6ppm range. All methyl esters were found to be unstable and their handling required special precautions.*

Characteristic ¹H and ¹³C NMR data of the various unsaturated functionalities which are embodied in one or more of compounds 1-6 are summarized in Figure 1. The proton NMR lines were fully assigned by double irradiation experiments and homonuclear shift correlation 2D ¹H-NMR (COSY-45) spectra². The latter experiments served not only for the line assignment of the unsaturated moieties but also for the determination of the methylene links between the functional sites (Figure 2).

A typical COSY-45 experiment is shown in Figure 3 for compound 2. (e.g. see the double quartet of H-11 at δ 2.12, $J_{10,11} \approx J_{11,12} \approx 7\text{Hz}$ and $J_{9,11} = 1.5\text{Hz}$). The six acids which were fully characterized are: 18-bromo(9E,17E)-octadeca-9,17-dien-7,15-diyneic acid(1), 18,18-dibromo(9E)-octadeca-9,17-dien-5,7-diyneic acid(2), 16-bromo(7E,11E,15E)-hexadeca-7,11,15-trien-5,13-diyneic acid(3), 16-bromo(7E,11E,15Z)-hexadeca-7,11,15-trien-5,13-diyneic acid(4), 16-bromo(7E,15E)-hexadeca-7,15-dien-5,13-diyneic acid(5) and 9,9-dibromonona-8-enoic acid(6) (Figure 2).

*Due to the instability of the methyl esters, the relative isolated amount of the acids are by no means their ratio in the sponge.

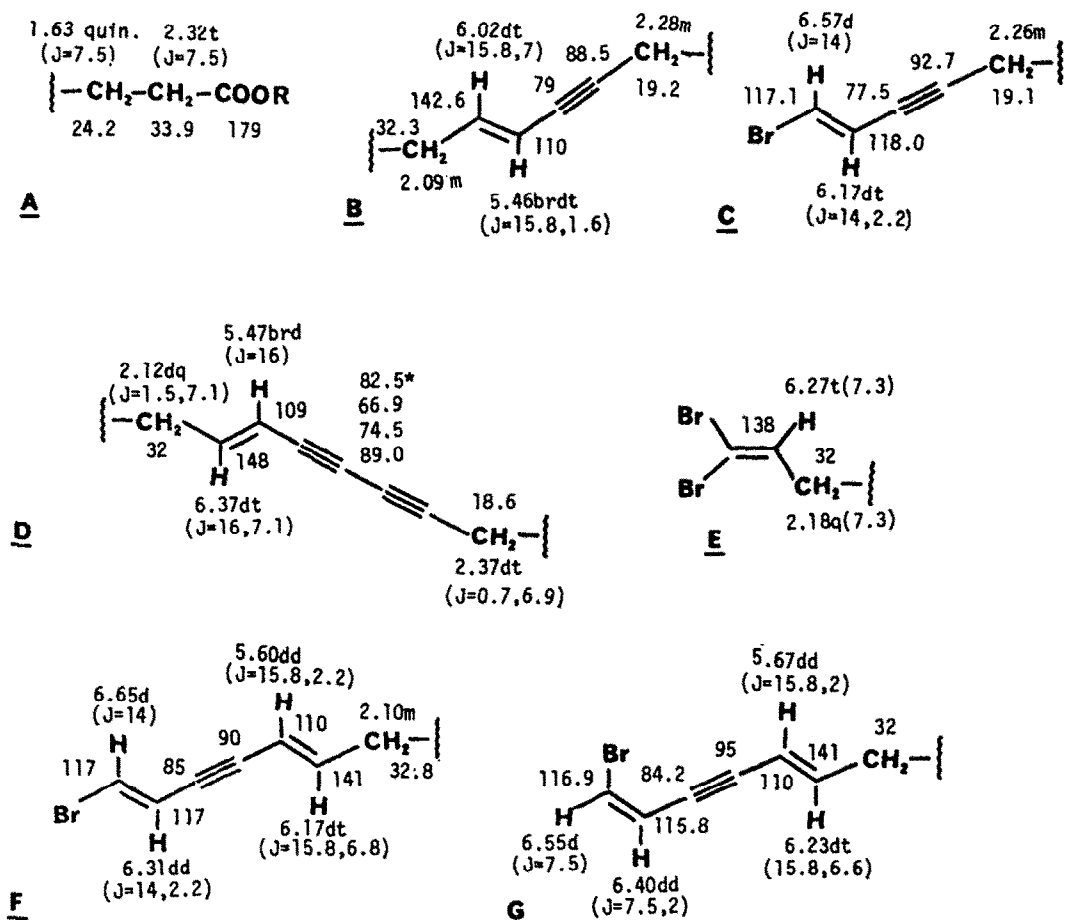


Figure 1. The functionalities of compounds 1-6.

The values of moieties **A**, **B** and **C** are according to compound 1, **D** and **E** according to compound 2, **F** according to compound 3 and **G** according to compound 4. The above values, with probable small deviation, are good representatives for the identification of these sites in other compounds.

Compound 1 $C_{18}H_{23}BrO_2$ possesses the partial structures **A-C** (Fig. 1) which are embodied in an octadecanoic acid.^{**} The structure of 1 was unequivocally confirmed by a COSY experiment and also by CH-correlation and HETCOSY³ experiments. The latter two experiments enabled the full carbon line assignment (see Experimental). Compound 1 was found to be identical with the 18-bromooctadeca-9(E),17(E)-dien-7,15-dienoic acid isolated from *Xestospongia testudinaria*.¹

The methyl ester of a second C_{18} -acid, compound 2b, $C_{19}H_{24}Br_2O_2$ possesses the typical fine structured UV absorption of an endiayne chromophore⁴ (partial structure **D**, Figure 1). The ¹H-NMR data of 2 (Figure 1) confirmed unequivocally moiety **D** ($J_{4,9} = 0.7\text{Hz}$) and also the two termini of the molecule, that is, the existence of the carboxylic site **A** on one end and the 1,1-dibromovinyl terminus on the other end. The latter moiety was clear from: (a) the absence, in the NMR spectrum of 2, of other end groups except for **A** and the observation, on the other hand, of a triplet of a vinyl proton (H-17) and (b) a $Br_2C=CHCH_2^+$ fragment (m/e 197/199/201 with relative intensities of 1,2,1) in the mass spectrum. A COSY-45 experiment (Figure 3) assigned all the protons of the molecule and determined, thereby, the mutual relationship of the three functionalities **A**, **D** & **E** (Figure 2). Compound 2 is unique among the known marine fatty acids for its dibromovinyl terminus.

^{**}Carbon chemical shifts of 19ppm and 32ppm, (for propargylic and allylic methylenes respectively), assisted with the determination of the number and ratio of methylenes adjacent to double and/or triple bonds.

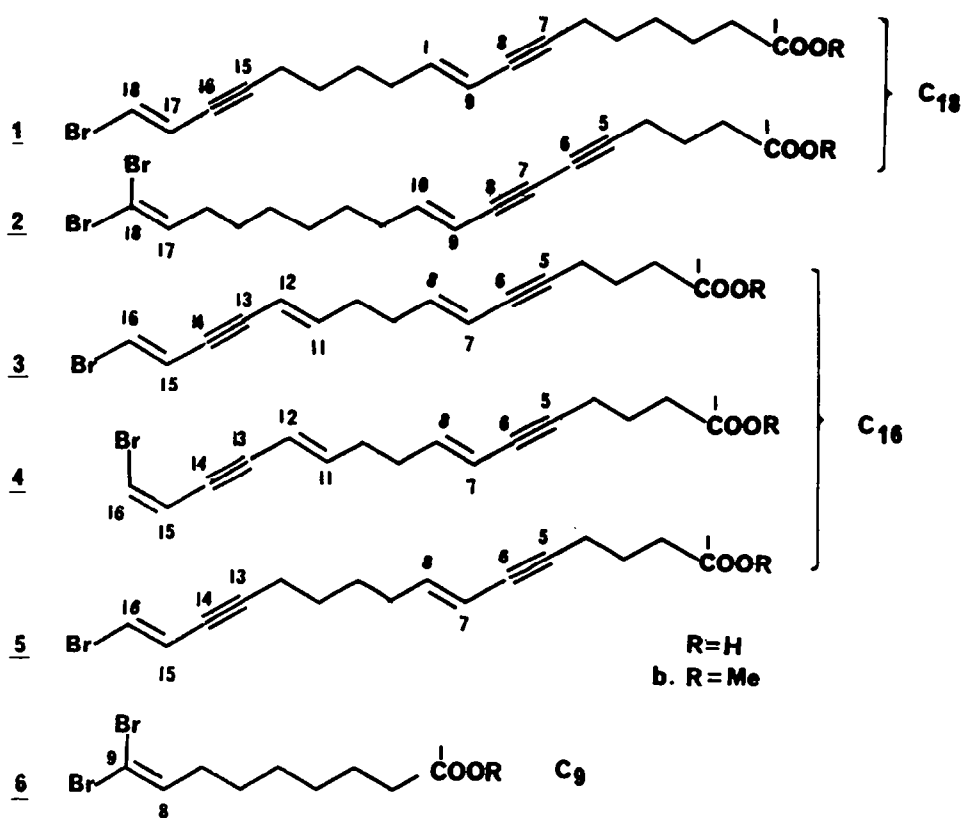


Figure 2. The six bromo unsaturated acids isolated from *Xestospongia* sp.

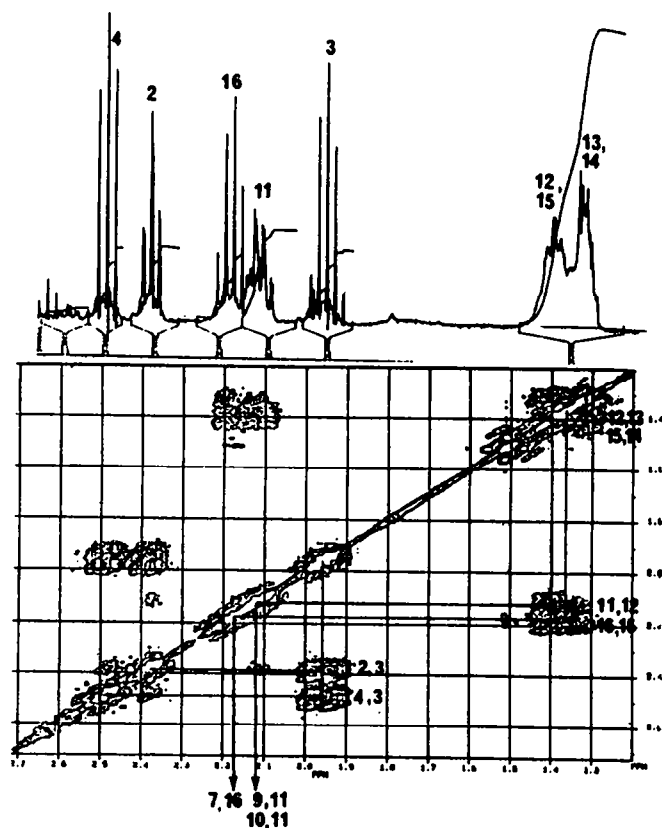


Figure 3. A partial COSY-45 spectrum of compound 2.

The NMR data of the third compound (**3**) $C_{17}H_{19}BrO_2$ (the methyl ester) suggested three partial structures **A**, **B** and **E** (Figure 1), which were confirmed and linked together, as shown in Figure 2, by a COSY-45 experiment (See Experimental for the full NMR data).

The fourth compound **4** was also a C_{16} -acid, $C_{17}H_{19}BrO_2$ (the methyl ester) and was found to be, as it turned out, an isomer of compound **3**. The only difference between compounds **3** and **4** was in the configuration of the bromovinyl terminus, that is, $15E$ in **3** and $15Z$ in **4** ($J_{15,16} = 14\text{Hz}$ and 7.5Hz respectively); compound **4** is therefore constructed from functionalities **A**, **B** and **G** (Figure 1) as was confirmed by a COSY-45 experiment.

The fifth compound (**5**), $C_{17}H_{21}BrO_2$ (the methyl ester) which was obtained in minute quantities only (ca. 85% pure) was found to be assembled from the same three functionalities as in compound **1**, namely, sites **A**, **B** and **C**. However, compound **5** is a C_{16} rather than a C_{18} -acid as confirmed by partial ^{13}C -NMR data (resonances of the protonated carbons only as measured from a polarization transfer experiment) and a COSY-45 experiment.

The last compound (**6**) $C_{10}H_{16}Br_2O_2$ (the methyl ester) is a nonanoic acid possessing the carboxylate end **A** on one side and the dibromovinyl terminus **E** on the other. The structure of compound **6** was confirmed by mass spectrum and carbon-NMR. Isolating of the latter acid is of interest from the biogenetic point of view as compound **6** might be a naturally oxidative degradation product of the C_{18} -acid **2**. The various compounds were found to possess moderate antibacterial activity against *B. subtilis* (the best MIC value, 25 $\mu\text{g/ml}$, being observed for compound **3**).

Experimental

IR Spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer and UV spectra on a Varian Cary 219 spectrophotometer. NMR spectra (in CDCl_3 or C_6D_6 with TMS as internal standard) were obtained with a Bruker AM-360 spectrometer equipped with an ASPECT 3000 computer. Mass spectra were recorded with a Finnigan 4020 quadrupole spectrometer equipped with a data system. Ionizing conditions for EI were 23-26eV and for $\text{Cl}(\text{NH}_3)$ 70eV.

Isolation of acids **1-6** from *Xestospongia* sp. The sponge was collected in the straits of the Gulf of Eilat in July 1984. Freeze-dried material (50gr) was ground and extracted with hot petroleum ether and then with CH_2Cl_2 to give after evaporation 2g of viscous dark oil. Repeated flash chromatographies on a silica gel-H column eluted with petroleum ether-ethylacetate and Sephadex LH-20 eluted with chloroform-hexane 7:3 afforded compounds **1**, **3** and **6** (50, 10 and 10 mg respectively from ca. 1g of extract).

Semi-purified extract (500mg) in ether (after two flash chromatographies on a silica gel-H column) was treated with a solution of CH_2N_2 in ether. The crude methyl esters were flash chromatographed as described above and then separated on a silica-gel 60 4mm ID HPLC column. (Compound **1b**, 100mg; compound **2b**, 5mg; compound **3b**, 50mg; compound **4b**, 2mg; compound **5b**, 2mg; compound **6b**, 2mg).

Compound **1**: CIMS m/e 370/368($\text{M}+\text{NH}_4^+$), EIMS 271($\text{M}-\text{Br}^+$); λ_{max} (MeOH): 235nm(18300); ν_{max} 3100, 2500br, 2220, 1715 cm^{-1} ; δ_{H} (CDCl_3): 6.57d(H-18, 14Hz), 6.17dt(H-17, 14, 2.2Hz), 6.02dt(H-10, 15.8, 7.0Hz), 5.46brdt(H-9, 15.8, 1.6Hz), 2.32t(H_2 -2, 7.5Hz), 2.28m(H_2 -6), 2.26m(H_2 -14), 2.09m(H_2 -11), 1.63quin. (H_2 -3, 7.5Hz), 1.61brs(H_4 -4, 5), 1.49brs(H_4 -12, 13); δ_{C} (CDCl_3): 179.7s(C-1), 142.6d(C-10), 118.0d(C-17), 117.1d(C-18), 110.3d(C-9), 92.7s(C-15), 88.5s(C-7), 79.3s(C-8), 77.5s(C-16), 33.9t(C-2), 32.3t(C-11), 28.4t, 28.3t(C-12, 13), 27.9t, 27.7t(C-4, 5), 24.2t(C-3), 19.2t(C-6), 19.16t(C-14).

Compound **2b**: CIMS m/e 447/445/443(MH^+ , methyl ester), 387/385/383($MH^+-C_2H_4O_2$); λ_{max} (heptane): 241 (3000), 254(4900), 268(6900), 284(5700)nm; ν_{max} 2900, 2220, 1730, 1100 cm^{-1} ; δ_H ($CDCl_3$): 6.37dt(H-10, 16.0, 7.1Hz), 6.27t(H-17, 7.2Hz), 5.47brd(H-9, 15.9Hz), 3.67s(H_3-OMe), 2.48t(H_2-2 , 7.4Hz), 2.37dt(H_2-4 , 6.9, 0.7Hz), 2.18q(H_2-16 , 7.2Hz), 2.12dq(H_2-11 , 7.1, 1.5Hz), 1.95quin. (H_2-3 , 6.9Hz), 1.40m($H_8-12-15$); δ_C ($CDCl_3$): 175.0s(C-1), 148.0d(C-10), 139.1d(C-17), 109.0d(C-9), 89.0s, 82.2s, 74.5s, 66.9s(C-5, 6, 7, 8), 51.6q(OCH_3), 32.9t, 32.8t, 32.2t(C-2, 11, 16), 28.5t(x2), 28.2t, 27.4t(C-12, 13, 14, 15), 23.4t(C-3), 18.6t(C-4).

Compound **3**: CIMS m/e 337/335(MH^+ , methyl ester), 255(MH^+-HBr), 165($MH^+-HBr-C_7H_6$); λ_{max} (heptane, methyl ester): 285(2800), 246(7600), 234(6300)nm; ν_{max} : 3600-2400br, 2220, 1715 cm^{-1} ; δ_H ($CDCl_3$): 6.65d(H-16, 14.0Hz), 6.31dd(H-15, 14.0, 2.2Hz), 6.17dt(H-11, 15.9, 6.8Hz), 6.04dt(H-8, 15.7, 6.8Hz), 5.60dd(H-12, 15.9, 1.8Hz), 5.46dt(H-7, 15.7, 2.0Hz), 2.51t(H_2-2 , 7.4Hz), 2.39dt(H_2-4 , 7.0, 2.0Hz), 2.21brs(H_4-9 , 10), 1.86quin. (H_2-3 , 7.3Hz); δ_C ($CDCl_3$): 178.8s(C-1), 144.3d, 141.7d(C-8, 11), 117.7d(x2)(C-15, 16), 110.9d, 110.1d(C-7, 12), 90.4s(C-13), 87.7(C-5), 84.9(C-14) 79.9(C-6), 32.8t, 32.4t, 31.9t(C-2, 9, 10), 23.7t(C-3), 18.8t(C-4).

Compound **4b**: CIMS m/e 337/335(MH^+), 255(MH^+-HBr), 165($MH^+-HBr-C_7H_6$), 101($C_5H_9O_2$); λ_{max} (heptane): 290(2200), 275(2700), 240(4000)nm; ν_{max} : 2200, 1725, 1350, 1150 cm^{-1} ; δ_H ($CDCl_3$): 6.55d(H-16, 7.5Hz), 6.40dd(H-15, 7.5, 2.0Hz), 6.23dt(H-11, 15.9, 6.6Hz), 6.04dt(H-8, 15.8, 6.6Hz), 5.67dd(H-12, 15.9, 1.9Hz), 5.47dt(H-7, 15.8, 2.0Hz), 3.65s(H_3-OMe), 2.43t(H_2-2 , 7.4Hz), 2.35dt(H_2-4 , 7.4, 2.0Hz), 2.21brs(H_4-9 , 10), 1.83quin. (H_2-3 , 7.3Hz); δ_C ($CDCl_3$): 180.0s(C-1), 144.6d, 141.7d(C-8, 11), 116.9d(C-15), 115.8d(C-16), 110.8d, 110.2d(C-7, 12), 95.9s, 84.2s(C-13, 14), 87.8s(C-5), 79.5s(C-6), 51.6q(OCH_3), 32.9t, 32.5t, 29.0t(C-2, 9, 10), 23.9t(C-3), 18.8t(C-4).

Compound **5b**: λ_{max} (heptane): 268(7000), 253(5500), 247(3000)nm; ν_{max} : 2900, 2390, 1750, 1600 cm^{-1} ; δ_H (C_6D_6): 6.56d(H-16, 14.1Hz), 6.25dt(H-15, 14.1, 2.2Hz), 6.15dt(H-8, 15.9, 7.1Hz), 5.48brd(H-7, 15.9Hz), 3.66s(H_3-OMe), 2.50t(H_2-2 , 7.4Hz), 2.40t(H_2-4 , 6.9Hz), 2.30dt(H_2-12 , 6.9, 2.2Hz), 2.05m(H_2-9), 1.98quin. (H_2-3 , 7.1Hz), 1.55m(H_2-11), 1.48m(H_2-10); δ_C ($CDCl_3$): 147.7d(C-8), 117.6d(x2)(C-15, 16), 109.7d(C-7), 51.1q(OCH_3), 32.7t(C-2), 30.4t(C-9), 27.94t, 27.92t(C-10, 11), 23.8t(C-3), 19.4t, 19.1t(C-4, 12).

Compound **6b**: CIMS 331/329/327(MH^+), 299/297/295(MH^+-CH_3OH), 217/215($BrC=C-(CH_2)_6-CO^+$), 189/187 (217/215-CO), 135(217/215-HBr), 107(189/187-HBr), 93, 71; λ_{max} (MeOH): 242(2100), 205(4500); ν_{max} : 3700, 3000br, 1700 cm^{-1} ; δ_H ($CDCl_3$): 6.38t(H-8, 7.2Hz), 2.35t(H_2-2 , 7.4Hz), 2.23q(H_2-7 , 7.2Hz), 1.64quin. (H_2-3 , 7.3Hz), 1.34brs(H_6-4 , 5, 6); δ_C of **6** ($CDCl_3$): 138.3d(C-8), 118.8s(C-9), 33.0t(C-2), 32.4t(C-7), 29.4t, 28.4t, 27.3t(C-4, 5, 6), 24.2t(C-3).

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